

EPO4/52028

PA 1224431

THE UNITED STATES OF AMERICA**TO ALL TO WHOM THESE PRESENTS SHALL COME:****UNITED STATES DEPARTMENT OF COMMERCE****United States Patent and Trademark Office**

September 17, 2004

REC'D 22 NOV 2004

WIPO PCT

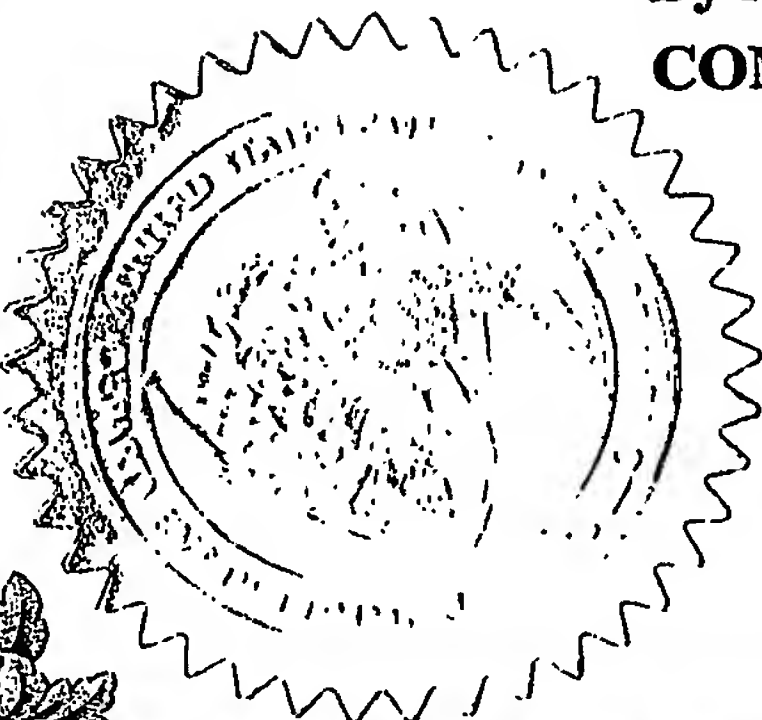
THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/508,486

FILING DATE: October 03, 2003

**PRIORITY
DOCUMENT**SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS



P. R. GRANT
Certifying Officer

Best Available Copy

PROVISIONAL APPLICATION FOR PATENT COVER SHEET
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. **EF195556615US**

00746 U.S. PTO
607508486



INVENTOR(S)				
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)		
Paul	Stoffels	Hoogstraten, Belgium		
Additional inventors are being named on the _____ separately numbered sheets attached hereto				
TITLE OF THE INVENTION (500 characters max)				
COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A NUCLEOSIDE RT INHIBITOR				
Direct all correspondence to: CORRESPONDENCE ADDRESS				
<input checked="" type="checkbox"/> Customer Number	000027777	Place Customer Number Bar Code Label here		
OR Type Customer Number here				
<input type="checkbox"/> Firm or Individual Name				
Address				
Address				
City	State	ZIP		
Country	Telephone	Fax		
ENCLOSED APPLICATION PARTS (check all that apply)				
<input checked="" type="checkbox"/> Specification	Number of Pages	18	<input type="checkbox"/> CD(s), Number	
<input type="checkbox"/> Drawing(s)	Number of Sheets		<input type="checkbox"/> Other (specify)	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76				
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT				
<input type="checkbox"/>	Applicant claims small entity status. See 37 CFR 1.27.			FILING FEE AMOUNT (\$)
<input type="checkbox"/>	A check or money order is enclosed to cover the filing fees.			
<input checked="" type="checkbox"/>	The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 10-0750/TIP0050/WIT			\$160.00
<input type="checkbox"/>	Payment by credit card. Form PTO-2038 is attached.			
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.				
<input checked="" type="checkbox"/>	No.			
<input type="checkbox"/>	Yes, the name of the U.S. Government agency and the Government contract number are: _____			

[Page 1 of 2]

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME

TELEPHONE

JESUS JUANOS I. TIMONE-DA

732 524 1513

Date

REGISTRATION NO.

(If appropriate)

Docket Number:

03/10/2003

43332

TIP0050

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Docket No. TIP-0050

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Paul Stoffels

For : COMBINATION OF A PYRIMIDINE CONTAINING NNTRI WITH A
NUCLEOSIDE RT INHIBITOR

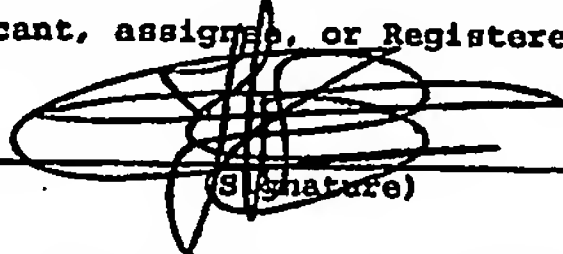
I hereby certify that this correspondence is being deposited with the
United States Postal Service as first class mail in an envelope addressed
to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

October 3, 2003

(Date)

Jesús Juanós i Timoneda

Name of applicant, assignee, or Registered Representative


(Signature)

October 3, 2003

(Date of Signature)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Enclosed find:

- Provisional Application for Patent Cover Sheet
- Application - 18 pages
- Express Mail Certificate
-

In the event of any non-payment of a required fee, the
Commissioner is authorized to charge or credit Deposit Account No.
10/0750/TIP005/JJiT in the name of Johnson & Johnson as required
to correct the error.

Respectfully submitted,


Jesús Juanós i Timoneda

Reg. No. 43,332

Attorney for Applicant(s)

Johnson & Johnson

One Johnson & Johnson Plaza

New Brunswick, NJ 08933-7003

(732) 524-1513

Date: October 3, 2003

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Applicant: Ppaul Stoffels

For : Combination of a Pyrimidine Containing Nntri With a
Nucleoside RT Inhibitor

Express Mail Certificate

"Express Mail" mailing number: EF-195556615US

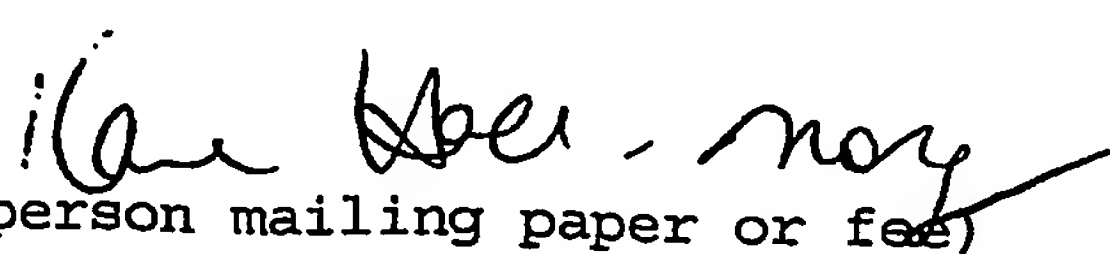
Date of Deposit: October 3, 2003

I hereby certify that this provisional application, including specification pages and claims; is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

A Combined Declaration and Power of Attorney will be submitted to the United States Patent and Trademark Office upon receipt of the U.S. Serial Number for this patent application.

Karen Hall-Morgan

(Typed or printed name of person mailing paper or fee)


(Signature of person mailing paper or fee)

COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A NUCLEOSIDE RT INHIBITOR

- 5 The present invention concerns the combination of a pyrimidine containing NNRTI with a nucleoside reverse transcriptase inhibitors useful for the treatment of HIV infected patients or for the prevention of HIV transmission or infection.

BACKGROUND OF THE INVENTION

- 10 Despite the fact that significant progress has been made by the introduction of HAART therapy (Highly Active Anti-Retroviral Therapy), resistance of the HIV virus against nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), protease inhibitors and even the more recent fusion inhibitors is still a major cause of
- 15 therapy failure. For instance, half of the patients receiving anti-HIV combination therapy do not respond fully to the treatment, mainly because of resistance of the virus to one or more drugs used. Moreover, it has been shown that resistant virus is carried over to newly infected individuals, resulting in severely limited therapy options for these drug-naïve patients. On the International AIDS Conference in Paris in July 2003
- 20 researchers released that the biggest study so far of resistance to AIDS drugs finds that about 10 percent of all newly infected people in Europe have drug-resistant strains. Smaller tests to determine the spread of resistance have been done in the high-risk city center of San Francisco. This test showed the highest level of resistance at 27 percent.
- 25 The pharmacokinetic profile of many commercially available antiretrovirals does not allow relatively low therapeutic doses. Poor pharmacokinetic profiles often in combination with poor solubility properties of the antiretrovirals cause the AIDS patient to face a high pill burden which is particularly undesirable for drug-naïve patients or first line therapy. Moreover, as a consequence of the AIDS virus resisting
- 30 even antiretroviral combination therapy, a physician will boost the plasma levels of the active drugs in order for said antiretrovirals to regain effectivity against the mutated HIV viruses. The consequence of which is an even higher increase in pill burden. Boosting plasma levels may also lead to an increased risk of non-compliance with the prescribed therapy and to increased side-effects.
- 35 Several attempts have been made to date to design combination regimens. For instance, the combination of lamivudine (a nucleoside RT inhibitor also named 3TC) at a 150 mg dose and zidovudine (a nucleotide RT inhibitor also named AZT) at a 300 mg dose,

formulated in an oral tablet and dosed twice daily, or the combination of abacavir sulphate at a dose equivalent to 300 mg abacavir (a nucleoside RT inhibitor), lamivudine at a 150 mg dose and zidovudine at a 300 mg dose, formulated in an oral tablet and dosed twice daily.

5

WO 93/23021 describes therapeutic combinations for the treatment of HIV-infections comprising zidovudine and an agent serving to enhance the antiviral activity against HIV populations otherwise resistant to zidovudine.

10 WO 96/01110 describes a triple combination of zidovudine, lamivudine and loviride. The latter being a non-nucleoside RT inhibitor of the α -APA class.

WO 03/016306 specifically discloses more than 250 pyrimidine derivatives having HIV replication inhibiting properties that act as non-nucleoside RT inhibitors

15 (NNRTIs) having the ability to inhibit the replication both wild-type and of mutant strains. WO 03/016306 also discloses the methods to synthesize these compounds. It further discloses combinations of said NNRTIs with other antiretrovirals, i.e. suramine, pentamidine, thymopentin, castanospermine, dextran (dextran sulphate), foscarnet-sodium (trisodium phosphono formate), zidovudine (3'-azido-3'-deoxythymidine, AZT), didanosine (2',3'-dideoxyinosine; ddI), zalcitabine (dideoxycytidine, ddC),
20 lamivudine (2'-3'-dideoxy-3'-thiacytidine, 3TC), stavudine (2',3'-didehydro-3'-deoxythymidine, d4T), abacavir, nevirapine (11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido-[3,2-b : 2',3'-e] [1,4]diazepin-6-one), efavirenz, delavirdine, TMC-120, TMC-125, tenofovir, (S)-8-chloro-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)-
25 imidazo-[4,5,1-jk] [1,4]benzodiazepine-2(1H)-thione, α -[(2-nitrophenyl)amino]-2,6-dichloro-benzene-acetamide, RO-5-3335, indinavir, ritonavir, saquinavir, lopinavir (ABT-378), nelfinavir, amprenavir, TMC-126, BMS-232632, VX-175, T-20, T-1249, AMD-3100 and hydroxyurea.

30 Notwithstanding existing combination therapy, there is still a need in the art for improved antiretroviral therapy, more particularly AIDS therapy. The need in the art is particularly acute for therapy that is effective not only on wild type HIV virus, but also on the increasingly more common resistant HIV viruses. It is thus highly desirable for especially first line therapy to design a combination regimen with a low pill burden that
35 limits or even suppresses the recurrence of drug resistant virus and which can be used and remains effective for a long term.

It is an object of the invention to provide a combination of more than one therapeutically effective antiretroviral drug which combination can be used as first line therapy in drug-naïve patients for a long period of time.

- 5 It is also an object of the invention to provide a combination of more than one therapeutically effective antiretroviral drug in which the antiretroviral drugs have a complementary resistance profile thus creating a high resistance barrier and thus allowing a drug-naïve patient to take the combination for a long period of time.
- 10 Another object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combination can be administered once daily thus reducing the pill burden for the patient.
- 15 A further object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein each of the active antiretroviral drugs of the combination can be co-formulated.

- 20 Yet a further object of the invention is to provide a combination of more than one therapeutically active antiretroviral drug wherein a therapeutically effective amount of each of the active antiretroviral drugs of the combination can be co-formulated in one single pharmaceutical formulation.

- 25 Another object of the present invention is to provide a combination of more than one active antiretroviral drug which combination can be used to prevent HIV transmission or infection in humans.

All references cited herein are incorporated by reference.

30 DESCRIPTION OF THE INVENTION

- It has been discovered that 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (herein named compound A) is a potent reverse transcriptase inhibitor that has an extremely high genetic barrier in combination with a favourable pharmacokinetic profile allowing once daily dosing. Based on this
- 35 discovery, a combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, characterized in that, compound (A) and the

nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

5 It was surprising to discover that compound (A) has all these properties together. This is unusual because one cannot predict what mutations will be selected in the HIV-1 genome by a given drug, whether the mutated virus will have any chance of survival under the pressure of the drug, how much drug is needed to limit or to suppress the recurrence of such mutated virus, and at what frequency such drug has to be given to maintain suppression of the development of a resistant virus that can break through the
10 genetic barrier of the drug.

Advantageously, the nucleoside reverse transcriptase inhibitor selects at least one mutation in the reverse transcriptase that does not cause resistance to compound (A). Therefore, in a preferred embodiment, a combination is provided comprising (i)
15 compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor, characterized in that, (1) compound (A) and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily and (2) the nucleoside reverse transcriptase inhibitor selects at least one mutation in the reverse
20 transcriptase that does not cause resistance to compound (A).

An interesting combination according to the present invention concerns a triple combination comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, (ii) a first nucleoside reverse
25 transcriptase inhibitor, and (iii) a second nucleoside reverse transcriptase inhibitor, characterized in that, compound (A) and the first and second nucleoside reverse transcriptase inhibitors are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

30 Even more interesting are those triple combinations wherein the first nucleoside reverse transcriptase inhibitor selects at least one mutation in the reverse transcriptase that does not cause resistance to compound (A), and the second nucleoside reverse transcriptase inhibitor selects at least one mutation in the reverse transcriptase that does not cause resistance to compound (A).

35 Compound (A) can also be used in a method for treating HIV infected patients that includes administering compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, in combination with one or two nucleoside reverse

transcriptase inhibitors, in which method a therapeutically effective amount of compound (A) and the one or two nucleoside reverse transcriptase inhibitors can be administered once daily. One embodiment of the present invention provides for the present combinations for use as a medicine. In another embodiment, the combinations of the present invention can be used in the manufacture of a medicament to treat HIV infected patients.

The present combinations are especially useful for the treatment of AIDS and related clinical conditions such as AIDS related complex (ARC), progressive generalised lymphadenopathy (PGL) or AIDS related neurological conditions such as multiple sclerosis. The present combination may be particularly useful for the treatment of drug-naïve HIV infected patients.

The present combinations are also useful for the prevention of HIV transmission or infection in humans, in particular sexual transmission. Thus, the present invention relates to the use of a combination according to the present invention for the manufacture of a medicament for the prevention of HIV infection or transmission via sexual intercourse or related intimate contact between partners. The invention also relates to a method of preventing HIV infection or transmission via sexual intercourse or related intimate contact between partners comprising administering to a subject in need thereof an effective amount of a combination according to the present invention.

In a preferred embodiment, each of the ingredients of a combination according to the present invention can be co-formulated in one pharmaceutical form and do not have to be administered in a separate pharmaceutical form. The daily therapeutic antiretroviral amount of the ingredients of a combination according to the present invention of such co-formulated single pharmaceutical form may then be given in a single unit dosage form or even in multiple unit dosage forms, such as two, three, four, five or even more unit dosage forms. A physician will be able to determine the exact dosage to be given taking into account the severity of the patient's condition as well as the patient's weight, gender and possibly other parameters such as individual differences in absorption, biodistribution, metabolism and excretion rates for each drug as well as other factors known to those skilled in the art.

Thus, in one embodiment, a pharmaceutical composition is provided comprising a pharmaceutically acceptable carrier and as active ingredients (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor.

In another embodiment, a pharmaceutical composition is provided comprising a pharmaceutically acceptable carrier and as active ingredients (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, (ii) a first
5 nucleoside reverse transcriptase inhibitor, and (iii) a second nucleoside reverse transcriptase inhibitor.

Preferred nucleoside reverse transcriptase inhibitors that can be used in the present combinations and methods employing such combinations include abacavir or a
10 pharmaceutically acceptable salt thereof, emtricitabine, racemic FTC and lamivudine (also named 3TC).

Emtricitabine or (-)-FTC is the left (-) rotatory enantiomeric form of racemic FTC or (\pm)-cis-4-amino-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-
15 pyrimidinone (FTC). It is a commercially available nucleoside analogue and exhibits activity against HIV-1 [Hoong et al. *Journal of Organic Chemistry* 1992 (5563-5565); Jeong et al *Journal of Medicinal Chemistry* 1993, 36 :2 (181-195); Van Roey et al. *Antiviral Chemistry and Chemotherapy* 1993, 4 :6 (369-375)]. The in vitro anti-HIV-1 activity of (-)-beta-enantiomer of FTC was reported to be 20-fold more than the (+)-
20 beta-enantiomer, and the (+)-enantiomer was significantly more toxic than the (-)-enantiomer to myeloid progenitor cells [Schinazi et al *Antimicrobial Agents and Chemotherapy* 1992, 36 :11 (2423-2431)]. The potential for HIV-1 resistance to FTC was evaluated by serial passage of the virus in human PBMCs and MT-2 cells in the presence of increasing drug concentrations. Highly drug-resistant HIV-1 variants
25 dominated the replicating virus population after two or more cycles of infection. RT derived from drug-resistant viral particles was 15- to 50-fold less susceptible to the 5'-triphosphate of FTC compared with the enzyme from parental drug susceptible virus. DNA sequence analysis of the RT gene amplified from resistant viruses consistently identified mutations at codon 184 from Met (ATG) to Val (GTG or GTA)
30 [Schinazi et al *Antimicrobial Agents and Chemotherapy* 1993, 37 :4 (875-881); Tisdale et al *Antiviral Research* 1993, 20 :Suppl 1; Smith et al *Journal of Virology* 1997, 71 :3 (2357-2362); Harrer et al *Journal of Infectious Diseases* 1996, 173 :2 (476-479); Tisdale et al *Proceedings of the National Academy of Sciences of the United States of America* 1993, 90 :12 (5653-5656)]. Due to this observed single
35 mutation in the YMDD of reverse transcriptase in the HIV-infected patients, (-)-FTC is not suitable for monotherapy and needs to be administered in combination with other antiretroviral agents to effectively treat patients infected with HIV. Emtricitabine is available as 200 mg capsules to be taken once a day.

Lamivudine has the chemical name (-)-2',3'-dideoxy-3'-thiacytidine and is described for instance in EP 382,526 as an antiviral nucleoside analogue. It is also a well established and useful antiretroviral which is commercially available for instance as 150 mg oral tablets. Lamivudine is also commercially available in combination with zidovudine (300 mg zidovudine / 150 mg lamivudine), and in combination with lamivudine and abacavir sulfate (300 mg zidovudine / 150 mg lamivudine / equivalent of 300 mg abacavir).

Abacavir is a well established and useful antiretroviral which is commercially available for instance as an oral solution of abacavir sulfate in a strength equivalent to 20 mg abacavir base/ml, or as an oral tablet of abacavir sulfate in a strength equivalent to 300 mg abacavir base. Abacavir sulfate is also commercially available in combination with lamivudine and zidovudine (300 mg zidovudine / 150 mg lamivudine / equivalent of 300 mg abacavir).

Abacavir is a carbocyclic nucleoside with potent and selective anti-HIV activity.

Abacavir in its optically active form is disclosed in EP-00434450. The cis-isomer of abacavir with unspecified absolute stereochemical configuration is described in EP-

349242. Abacavir is one of the most potent NRTI developed to date. An average reduction in viral load of more than 1.4 log₁₀ RNA copies/ml is observed after a short course of abacavir monotherapy. In vitro, resistant virus is not rapidly selected by abacavir. A significant decrease in susceptibility to abacavir in wild-type or zidovudine-resistant HIV-1 strains was not observed until after eight to ten passages in MT-4 cells. A set of resistance mutations at HIV reverse transcriptase (RT) codons, 65R, 74V, 115F and/or 184V, are selected during in vitro passage with abacavir, and a combination of these mutations was required to confer a 10-fold reduction in abacavir susceptibility in a laboratory strain of HIV. The first mutation detected upon passage of HIV-1 in an increasing concentration of abacavir is M184V, which confers only a 3-fold decrease in HIV-1 susceptibility. Phenotype resistance to 3TC and/or the presence of the 184V mutation does not prevent viral load response to abacavir therapy.

Resistance to multiple nucleosides is associated with a decreased or absent response to abacavir [Kumar et al *Antimicrobial Agents and Chemotherapy* 1999, 43:3 (603-

608); Lanier et al *International Conference on Retroviruses and Opportunistic*

Infections 1998, 5th:Chicago Posted on: 16 April 1999].

In a preferred embodiment, a combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii)

lamivudine, characterized in that, compound (A) and lamivudine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

5 In another preferred embodiment, a combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) emtricitabine, characterized in that, compound (A) and emtricitabine are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

10 In another preferred embodiment, a combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) abacavir or a pharmaceutically acceptable salt thereof, characterized in that, compound (A) and abacavir are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

15 In another preferred embodiment, a combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) abacavir sulfate, characterized in that, compound (A) and abacavir sulfate are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

20 In another preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) lamivudine, and (iii) abacavir or a pharmaceutically acceptable salt thereof, characterized in that, compound (A) and lamivudine and abacavir are therapeutically effective HIV inhibitors at a dose that can be administered once daily.

25 In another preferred embodiment, a triple combination is provided comprising (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) lamivudine, and (iii) abacavir sulfate, characterized in that, compound (A) and lamivudine and abacavir sulfate are therapeutically effective HIV inhibitors at
30 a dose that can be administered once daily.

The following preferred triple combinations are also included

- (a) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug with emtricitabine and lamivudine;
- 35 (b) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug with emtricitabine and abacavir or a pharmaceutically acceptable salt thereof.

A preferred form of compound (A) is the E-isomer, i.e. (E)- 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile (hereinafter called compound E-(A)). The Z-isomer of compound (A) (hereinafter called compound Z-(A)) has relatively high potency against wild-type HIV-1, and also finds use in the present triple combinations. However, it is less active against single and double mutants in comparison to the E-isomer. Table 1 shows the IC₅₀ value in nM of the E and Z-isomer of compound A.

Table 1.

HIV RT mutation	E-isomer	Z-isomer
Wild-type	0.4	0.6
100I	0.4	6.3
103N	0.3	1.6
181C	1.3	5.0
188L	2.0	32
227C	2.0	4.0
100I+103N	7.9	790
103N+181C	1.0	40
227L+106A	1.0	4.0

10

The respective daily dose for each of the compounds of a combination according to the present invention may range between 10 mg and 800 mg, preferably between 50 and 400 mg, more preferably between 50 and 300 mg. In particular, the daily dose for compound E-(A) may range between 10 mg and 500 mg, preferably between 10 and 300, more preferably between 50 and 250 mg.

15

The weight ratio of each couple of active components of a combination according to the present invention taken on a daily basis may vary in a range from 1/10 to 10/1. Suitably, the weight ratio of each couple varies between 1/6 and 6/1, possibly between 1/4 and 4/1, even between 1/3 and 3/1, and also even between 1/2 and 2/1.

20

Table 2 lists some examples of the daily dose for each of the active ingredients in combinations of compound E-(A), abacavir and lamivudine wherein the dose mentioned in the table for abacavir sulfate is the equivalent dose of abacavir base.

25

Active Ingredient	combination 1	combination 2	combination 3
Compound E-(A)	50 mg	100 mg	200 mg
Lamivudine	150 mg	150 mg	150 mg
Abacavir sulfate	300 mg	300 mg	300 mg

Thus, an interesting combination according to the present invention comprises compound E-(A) in a daily dose ranging between 10 mg and 500 mg, a daily dose of 150 mg lamivudine and a daily dose of an equivalent of 300 mg abacavir base.

5 Suitably, such combination is formulated in a single pharmaceutical form.

Another interesting combination according to the present invention comprises compound E-(A) in a daily dose ranging between 50 mg and 250 mg, a daily dose of 150 mg lamivudine and a daily dose of an equivalent of 300 mg abacavir base.

10 Suitably, such combination is formulated in a single pharmaceutical form.

The compounds of the present combinations may be administered simultaneously, concurrently or sequentially. Simultaneous administration may be done by employing a unitary pharmaceutical formulation or separate pharmaceutical formulations. In
15 general, the combinations may be administered by topical, oral, rectal, intravenous, subcutaneous or intramuscular routes. For first line therapy of HIV infection, simultaneous administration employing a unitary pharmaceutical formulation is preferred.

20 The present invention also relates to a pharmaceutical composition in a form adapted to be applied to a site where sexual intercourse or related intimate contact can take place, such as the genitals, rectum, mouth, hands, lower abdomen, upper thighs, especially the vagina and mouth, comprising a pharmaceutically acceptable carrier and as active ingredients an effective amount of a triple combination according to the present
25 invention. As appropriate special adapted compositions there may be cited all compositions usually employed for being applied to the vagina, rectum, mouth and skin such as for example gels, jellies, creams, ointments, films, sponges, foams, intravaginal rings, cervical caps, suppositories for rectal or vaginal application, vaginal or rectal or buccal tablets, mouthwashes. To prepare such pharmaceutical compositions, an
30 effective amount of each of the particular compounds of the triple combination as the active ingredients is combined in intimate admixture with a pharmaceutically acceptable carrier, which carrier may take a wide variety of forms depending on the form of administration. In order to increase the residence time of such pharmaceutical composition at the site of administration, it may be advantageous to include in the
35 composition a bioadhesive, in particular a bioadhesive polymer. A bioadhesive may be defined as a material that adheres to a live biological surface such as for example a mucus membrane or skin tissue.

In one aspect of the invention, the present combinations can be formulated in an oral tablet form further comprising pharmaceutically acceptable excipients having a weight ranging between 150 mg and 600 mg, suitable ranging between 200 and 400 mg.

- 5 Convenient oral tablet forms containing the active ingredients according to the present invention have a total nominal weight ranging between 200 mg and 1500 mg, suitably between 500 mg and 1250 mg, more suitable between 600 and 1100 mg.

10 Thus, the present invention also relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and as active ingredients an effective amount of each of the compounds of the present combination characterized in that the pharmaceutical composition is bioadhesive to the site of application. Preferably, the site of application is the vagina, rectum, mouth or skin, most preferred is the vagina.

- 15 Pani A *et al* in Antiviral Chemistry & Chemotherapy (2001), 12(Suppl. 1), 51-59 described the ability of lamivudine to delay the viral breakthrough.

In order to demonstrate the ability of compound A to prevent HIV infection via sexual intercourse or related intimate contact between partners, compound A can be tested in
20 the following test. Immature monocyte derived dendritic cells (immMO-DC) represent a good model for interstitial dendritic cells, which are early targets during sexual HIV transmission and important initiators of the immune response. These immMO-DC were used in "in vitro" models to test the prevention of HIV infection via sexual intercourse or related intimate contact between partners. One such model is described
25 in the experimental part and indicates that the compound A potently inhibits HIV replication in MO-DC/ CD4(+) T cell co-cultures.

EXPERIMENTAL PART

Pharmacokinetics of compound E-(A)

A double-blind, randomized, placebo-controlled Phase I trial was designed to evaluate safety, tolerability, and *ex-vivo* pharmacokinetics of single doses of compound E-(A) in healthy male volunteers. Oral doses of 12.5, 25, and 50 mg were formulated in PEG
30 400 and taken with a standard meal. The pharmacokinetic results are shown in Table 3.

The pharmacokinetic results of another double-blind, randomized, placebo-controlled Phase I study with 4 dosing sessions to evaluate the safety, tolerability, pharmacokinetics and *ex-vivo* pharmacodynamics of single 100 mg and 200 mg oral doses of compound E-(A) in healthy male subjects are also reported in Table 3. Randomization was such that for each session 6 subjects received the same dose of

compound E-(A) and 3 subjects received placebo. There was a time interval of about 14 days between each dosing session

Table 3 shows that high and dose-proportional exposures were obtained. The correlation coefficient for the 5 C_{max} datapoints is 0.9897 and for the area under the curve values between 0 and 48 hours (AUC_{0-48hr}) 0.9952. Half-life of plasma concentrations ranged between 37 and 39 hours. The compound was well tolerated by the volunteers. No relevant adverse effects of the drug were noted.

Table 3

Parameter	12.5 mg	25 mg	50 mg	100 mg	200 mg
C_{max} (ng/ml)	73±14	149±32	267±27	482±121	807 ±207
T_{max} (hr)	4.0±0	4.0±1.3	4.0±1.3	4.3±0.8	4.3 ±0.8
AUC_{0-48hr} (nghr/ml)	1337±310	2805±496	5094±509	8162±2251	15592±2746
$AUC_{0-∞}$ (nghr/ml)	2210±473	4637±1164	8872±1342	15844±4592	
$T_{1/2}$ (hr)	37.1	38.7	45±9	55±18	

Virological profile of Compound E-(A)

- 5 Compound E-(A) was tested in a cell-based assay, using natural host cells of HIV. MT-4 cells (a cell line of human T cells) were infected with HIV-1 (wild type or mutants) and exposed to different concentrations of antiviral compound in the presence of 10% fetal calf serum. Cytotoxicity was determined in parallel with the antiviral activity so that the selectivity of the antiviral effect could be assessed. Active compounds have to
- 10 penetrate the cell membrane in order to interfere with replication steps inside the cell. After four days of incubation at 37°C, the viability of the HIV and mock-infected cells was assessed by an automated tetrazolium-based colorimetric assay. This method enabled the calculation of both the 50% inhibitory concentration for inhibition of viral cytopathicity (IC50), the IC90, and the 50% cytotoxic concentration (CC50). The ratio
- 15 CC50/IC50, also called the selectivity index, is an indication of the specificity of the antiviral effect. Tested HIV strains included: Wild type (wt) HIV-1; a panel of single and double mutants; obtained by site-directed mutagenesis (SDM), and a panel of clinical isolates, selected for resistance against NNRTIs.
- 20 *Activity towards wild type and SDM mutants*
A limited panel of HIV-1 mutants was constructed using site-directed mutagenesis (SDM) and homologous recombination techniques. Compound E-(A) was tested against an extended panel of single and double mutants known to be resistant against

commercially available NNRTIs. Nevirapine (NVP) and efavirenz (EFV) were included as controls.

The results are shown in Table 4 (values presented are IC₅₀ values in nM). For wild type virus, the obtained IC₅₀ was 0.4 nM (0.15 ng/ml) and the IC₉₀ 1.3 nM (0.48 ng/ml). The HIV strain with the lowest sensitivity against compound E-(A) within this selection was the double mutant 100I+103N, with an IC₅₀ of about 8 nM and an IC₉₀ of about 16 nM.

10 Table 4:

	NVP	EFV	Compound E-(A)
wild type	81	1.4	0.4
100I	597	35	0.4
101E	547	5	1.6
103N	2,879	28	0.3
106A	2,983	23	0.2
108I	-	2	0.3
138K	64	1.3	0.4
179D	161	6	0.6
179E	158	5	0.4
181C	10,000	2	1.3
188C	3,764	5	0.1
188H	241	9	0.2
188L	10,000	78	2.0
190A	4,101	8	0.3
190S	10,000	275	0.1
225H	171	2	0.3
227C	1,816	36	2.0
227L	78	0.3	0.3
234I	45	NT	0.3
236L	41	1	0.3
100I+103N	10,000	10,000	7.9
101E+103N	7,033	84	0.5
103N+181I	10,000	37	1.0
227L+106A	10,000	8	1.0

Development of resistance in vitro

NNRTIs are highly selective inhibitors of HIV-1 but their current clinical use is limited by the rapid emergence of NNRTI (cross-) resistance. The rate of resistance emergence

against compound E-(A) and the first generation NNRTIs nevirapine and efavirenz was compared in vitro.

MT4 cells were infected with wild type HIV-1 at high multiplicity of infection (>1 infectious virus per cell, to maximize the genetic diversity of the virus population) in the presence of various concentrations of compound E-(A) (40, 200, 1000 and 5000 \times IC₅₀), and were monitored twice a week for virus replication. Emerging virus was collected for pheno- and genotyping. Cultures without evidence of virus replication were further sub-cultivated in the presence of the same concentration of inhibitor for a total duration of 30 days (10 passages).

Resistance to nevirapine emerged within 3-6 days, at all tested concentrations.

Breakthrough virus harboured the typical Y181C mutation. The same experiments with efavirenz resulted in the selection of G190E at all concentrations (up to 5 μ M) within 3 to 7 days. Compound E-(A) did not select for resistant virus within 30 days using wild-type virus. If a double resistant mutant K103N+Y181C (IC₅₀ 0.8 nM) was used instead of wild type virus, resistance did emerge at all tested concentrations. Starting from the single mutants Y181C (IC₅₀ 1.3 nM) or 103N (IC₅₀ 0.3 nM), virus breakthrough did not occur at 40 and 200 nM, but did occur at 10 nM.

In this experimental setting of high genetic diversity, HIV-1, resistant to first generation NNRTIs, was selected very rapidly. Resistant viruses harboured only one mutation. In contrast, emergence of HIV-1, resistant to compound E-(A) was delayed or did not occur.

Cardiovascular and pulmonary safety of compound E-(A)

Compound E-(A) had little or no effect on cardiovascular and pulmonary parameters in vivo at plasma levels covering and exceeding the targeted plasma levels in man and at concentrations in vitro covering or exceeding the anti-viral concentration in vitro.

In vitro models to test the ability of compound E-(A) to prevent HIV infection via sexual intercourse or related intimate contact between partners.

For instance, in one model, monocyte-derived dendritic cells (MO-DC) were infected for 2 hours with the monotropic HIV strain Ba-L at a multiplicity of infection (MOI) of 10^{-3} . After infection, cells were washed 6 times and resuspended in 10% BCS at 400.000 cells/ml. Autologous CD4(+) T cells were purified out of the lymphocyte fraction of the same elutriation as the MO-DC and used at a concentration of 2×10^6 cells/ml (ratio MO-DC/CD4(+) T : 1/5).

A serial dilution of a compound of formula (I) (test compound) was added to the MO-DC/ CD4(+) T cell co-cultures. Each experiment was done in 96-well plates, in which

each cup contained 50µl of MO-DC, 50µl of CD4(+) T cells and 100µl of test compound.

Half of the culture medium, with test compound, was refreshed twice weekly.

Supernatants were analysed in ELISA after 14 days of culture. To determine antiviral

activity, the test compound concentration able to suppress 50% of the viral replication at

5 the end of the primary cultures (EC50) was measured. For compound E-(A), the EC50 value was 0.55 nM.

CLAIMS

1. A combination comprising
 - (i) 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]-amino]-2-pyrimidinyl]-amino]-benzonitrile, also named compound (A), or a stereoisomeric form or a pharmaceutically acceptable salt or a prodrug thereof, and
 - (ii) a nucleoside reverse transcriptase inhibitor, characterized in that, compound (A) and the nucleoside reverse transcriptase inhibitor are therapeutically effective HIV inhibitors at a dose that can be administered once daily.
2. A combination according to claim 1 wherein compound (A) occurs in its E-isomeric form.
3. A combination according to claims 1 or 2 wherein the nucleoside reverse transcriptase inhibitor selects at least one mutation in the reverse transcriptase that does not cause resistance to compound (A).
4. A combination according to any one of claims 1 to 3 wherein the combination comprises a second nucleoside reverse transcriptase inhibitor wherein also the second nucleoside reverse transcriptase inhibitor is a therapeutically effective HIV inhibitor at a dose that can be administered once daily.
5. A combination according to any one of claims 1 to 4 wherein the first and optionally second nucleoside reverse transcriptase inhibitor is abacavir or a pharmaceutically acceptable salt thereof, emtricitabine, racemic FTC or lamivudine (also named 3TC).
6. A combination according to any one of claims 1 to 5 wherein the first nucleoside reverse transcriptase inhibitor is abacavir or a pharmaceutically acceptable salt thereof and the second nucleoside reverse transcriptase inhibitor is lamivudine.
7. A combination according to any one of claims 1 to 6 wherein the weight ratio of each couple of components of the combination taken on a daily basis may vary in a range from 1/6 to 6/1.
8. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and as active ingredients (i) compound (A) or its stereoisomeric form or pharmaceutically acceptable salt or its prodrug, and (ii) a nucleoside reverse transcriptase inhibitor.

9. A pharmaceutical composition as claimed in claim 8 further comprising a second nucleoside reverse transcriptase inhibitor.
10. A pharmaceutical composition as claimed in claim 9 comprising between 10 mg and 500 mg compound E-(A), an equivalent of 300 mg abacavir base and 150 mg lamivudine.
11. A combination as claimed in any one of claims 1 to 7 for use as a medicine.
12. Use of a combination as claimed in any one of claims 1 to 7 for the manufacture of a medicament for the prevention of HIV infection or transmission via sexual intercourse or related intimate contact between partners.

ABSTRACT

COMBINATION OF A PYRIMIDINE CONTAINING NNRTI WITH A
NUCLEOSIDE RT INHIBITOR

5

The present invention concerns the combination of a pyrimidine containing NNRTI
with a nucleoside reverse transcriptase inhibitor, useful for the treatment of HIV
infected patients or for the prevention of HIV transmission or infection. It further
10 relates to pharmaceutical formulations containing such combinations.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☐ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.